WS1.5 Restoration of the CFTR function by antisense oligonucleotide splicing modulation

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10–15% of mutations in the CFTR gene affect its correct splicing. The disease severity in patients carrying these mutations is highly variable, correlated with the level of aberrantly spliced transcripts. We aim to develop a splicing mutation-specific therapy using antisense oligonucleotides (AOs), to modulate the splicing pattern in patients carrying common splicing mutations. We focused on a common splicing mutation, the 3849+10kb C-to-T, which leads to inclusion of an 84 bp cryptic exon between exons 22–23 in the mature mRNA. This cryptic exon contains an in-frame stop codon that leads to degradation of a significant fraction of the mRNA by the NMD pathway as well as to the production of prematurely truncated nonfunctional proteins. We designed 4 2′-O-methyl phosphorothioate-modified AOs, targeted to prevent the recognition of enhancer splice motifs in the cryptic exon or to mask the junctions between this exon and its flanking sequences. Epithelial cell line established from a nasal polyp of a CF patient carrying the 3849+10kb C-to-T mutation was transfected with 2 of the designed AOs. AOs-treated cells showed a highly significant decrease in the level of aberrantly spliced CFTR mRNA, along with a significant increase in normal spliced CFTR mRNA levels, reflecting transcripts that under normal conditions are degraded by the NMD pathway. These results indicate that AOs targeted to mask splicing motifs around the cryptic exon generated due to the 3849+10kb C-to-T splicing mutation, can modulate increase the correct splicing. Further studies are required to investigate whether these AO_S can restore the CFTR function and improve patients' clinical state.

WS1.6 OligoG normalizes the CF mucus phenotype

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Objectives: Cystic fibrosis (CF) is a recessive genetic disease caused by non-functional chloride and bicarbonate ion transport via CFTR. Mucus stagnation occurs because the mucociliary clearance system is compromized, leading to lung infections and damage. Using an explant system, we found that, in contrast to normal mucus, the mucus of the small intestine is attached to the epithelium in mice with a non-functional CFTR (CftrΔF508). This could be reverted to a non-attached phenotype by 100 mM bicarbonate.
OligoG CF-5/20 is a natural product derived from alginate comprised of mainly guluronate oligomers, with average length of 13 monomers. Previous studies have shown that this oligomer alters the rheology of mucin/alginate gels, mucin/DNA gels and CF sputum. Because of this, OligoG is being tested as an inhalation therapy on CF patients. We have now tested OligoG on mouse ileum CF mucus.
Methods: Explants from the small intestine of CftrΔ508 mutant mice were mounted in the horizontal Ussing-type chamber. OligoG (1%, 1.2%, 1.5%, 2%, 3% or 6%) was added to the apical buffer, pH of 7.4. The attachment of the already formed mucus was assessed by comparing the total mucus thickness before and after aspiration.
Results: OligoG at 1.5% or higher normalized the mucus phenotype without increase in mucus thickness. At 1% OligoG the mucus remained attached and at 1.2% an intermediate phenotype was observed.
Conclusion: The effects are likely due to OligoG's known ability to chelate calcium. These observations indicate how OligoG could work in CF patients by normalizing mucus layers in both the gut and potentially the lungs, at therapeutically relevant concentrations.